

ONR FINAL REPORT

GRANT #: N00014-93-10229

PRINCIPAL INVESTIGATOR: Dr. William J. Lennarz

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GRANT TITLE: Formation of Calcite Biocrystals; Structure and Formation of Matrix Glycoproteins

REPORTING PERIOD: Final

AWARD PERIOD: 03/01/93 - 02/28/97

I. Studies in the Distribution of Protein in the Sea Urchin Spicule

The presence of proteins associated with the CaCO_3 -containing biocrystals found in a wide variety of marine organisms is well established. In these organisms, including the primitive skeleton (spicule) of the sea urchin embryo, the structural and functional role of these proteins either in the biominerization process or in control of the structural features of the biocrystals is unclear. Recently, one of the matrix proteins of the sea urchin spicule, SM30, has been shown to contain a carbohydrate chain (the 1223 epitope) that has been implicated in the process whereby Ca^{2+} is deposited as CaCO_3 . Because an understanding of the localization of this protein, as well as other proteins found within the spicule, is central to understanding their function, we undertook to develop methods to localize spicule matrix proteins in intact spicules, using immunogold techniques and scanning electron microscopy. Gold particles indicative of this matrix glycoprotein could not be detected on the surface of spicules that had been isolated from embryo homogenates and treated with alkaline hypochlorite to remove any associated membranous material. However, when isolated spicules were etched for 2 min with dilute acetic acid (10 mM) to expose more internal regions of the crystal, SM30 and perhaps other proteins bearing the 1223 carbohydrate epitope were detected in the calcite matrix. These results, indicating that these two antigens are widely distributed in the spicule, suggest that this technique should be applicable to any matrix protein for which antibodies are available.

II. Studies on the Role of Sea Urchin Done Morphogenetic Protein (suBMP) in Differentiation of Spicule Forming Primary Mesenchymal Cells

On going work in our laboratory has shown that suBMP, a bone morphogenetic protein 1 family member, is the homolog of the *Drosophila* embryonic patterning gene tolloid. Resequencing of the 3' end of the suBMP cDNAs has revealed an extended open reading frame with high homology to human and murin tolloid homologs. Recent studies in the laboratories of Darwin Prockop, Daniel Greenspan, and Effrat Kessler, have demonstrated that both human bone morphogenetic protein 1 and human tolloid act as procollagen C-terminal proteinases, releasing the C-terminal propeptide from procollagen. This proteolytic processing event is necessary for the deposition of collagen fibrils. We have been able to demonstrate the existence of a procollagen C-terminal proteinase (PCP) activity in *S. purpuratus* extracts containing suBMP. This PCP activity is heat labile, and

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demonstrates time dependent cleavage. Current studies are underway to determine if recombinant suBMP has PCP activity.

Previous studies in our lab and others have demonstrated that appropriate collagen processing is necessary for gastrulation and spiculogenesis to occur in the developing sea urchin embryo, as well as for calcium carbonate deposition into growing spicules in primary mesenchyme cell culture. Disruption of collagen hydroxylation or crosslinking blocks spicule growth. SuBMP may also play an important role in this process. By removing the C-terminal propeptide from triple-helical procollagen, the collagen triple helix is rendered insoluble, and can be deposited into growing collagen fibrils. If suBMP is responsible for the PCP activity in *S. purpuratus*, then its function will be essential for collagen deposition and therefore sea urchin development.

III. Publications Supported by the ONR Grant:

1. Cho, J.W., Partin, J.S. and Lennarz, W.J. (1996), A Technique for Detecting Matrix Proteins in the Crystalline Spicule of the Sea Urchin Embryo. *Proc. Natl. Acad. Sci.* 93, 1281-1286.
2. Brown, Martin, F., Partin, Jacqueline S., Killian, Christopher E. and Lennarz, William J. (1995), Spiculogenesis in the Sea Urchin Embryo: Studies on the SM30 Spicule Matrix Protein. *Develop. Growth and Differ.* 37, 69-78.
3. Lennarz, William J. (1994), Fertilization in Sea Urchins: How many Different Molecules are Involved in Gamete Interaction and Fusion. *Zygote* 2, 1-4.
4. Hwang, Sheng-Ping L., Partin, Jacqueline S. and Lennarz, William J. (1994), Characterization of a Homolog of Human Bone Morphogenetic Protein 1 in the Embryo of the Sea Urchin, *S. purpuratus*. *Development* 120, 559-568.
5. Hwang, Sheng-Ping L. and Lennarz, William J. (1993), Studies on the Cellular Pathway Involved in Assembly of the Embryonic Sea Urchin Spicule. *Exp. Cell Res.* 205, 383-387.

REPORT OF INVENTIONS AND SUBCONTRACTS				FORM APPROVED OMB NO. 0704 0016																					
<p>Pursuant to "Patent Rights" Contract Clause. See Instructions on Reverse Side.)</p> <p>1a. Name of Contractor/ Subcontractor The Research Foundation of SUNY</p> <p>1b. Address (include Zip Code) Office of Sponsored Programs SUNY at Stony Brook Stony Brook New York 11794-3366</p> <p>1c. Contract Number N0001493-10229</p> <p>1d. Award Date (yymmdd) 930301</p> <p>2a. Name of Government Prime Contractor Office of Naval Research</p> <p>2b. Address (include Zip Code)</p> <p>3. Type of Report (check one) <input checked="" type="checkbox"/> Final <input type="checkbox"/> Interim</p> <p>4. Reporting Period (yymmdd) From: 93/03/01 To: 97/02/28</p>																									
SECTION I - SUBJECT INVENTIONS																									
<p>5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR (If "None", so state)</p> <table border="1"> <tr> <td>a. Name of Inventor(s) (Last, First, M.I.)</td> <td>b. Title of Invention(s)</td> <td>c. Disclosure No. Patient application Serial No. or Patent No.</td> <td>d. Election to File Patient Applications</td> <td>e. Confirmatory Instrument or Assignment Forwarded To Contracting Officer</td> </tr> <tr> <td>None</td> <td></td> <td></td> <td>United States Yes</td> <td>Foreign No</td> </tr> <tr> <td></td> <td></td> <td></td> <td>Yes</td> <td>Yes</td> </tr> <tr> <td></td> <td></td> <td></td> <td>No</td> <td>No</td> </tr> </table>						a. Name of Inventor(s) (Last, First, M.I.)	b. Title of Invention(s)	c. Disclosure No. Patient application Serial No. or Patent No.	d. Election to File Patient Applications	e. Confirmatory Instrument or Assignment Forwarded To Contracting Officer	None			United States Yes	Foreign No				Yes	Yes				No	No
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<p>f. Employer of Inventor(s) Not Employed by Contractor/Subcontractor</p> <table border="1"> <tr> <td>i. Name of Inventor</td> <td>g. Elected Foreign Countries in which a Patent Application will be Filed</td> </tr> <tr> <td>ii. Name of Employer</td> <td>l. Title of Invention</td> </tr> <tr> <td>iii. Address of Employer (include zip code)</td> <td>ii. Foreign Countries of Patent Application</td> </tr> </table>						i. Name of Inventor	g. Elected Foreign Countries in which a Patent Application will be Filed	ii. Name of Employer	l. Title of Invention	iii. Address of Employer (include zip code)	ii. Foreign Countries of Patent Application														
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SECTION II - SUBCONTRACTS (Containing a "Patent Rights" clause)																									
<p>6. Subcontracts Awarded by Contractor/Subcontractor (If "None", so state)</p> <table border="1"> <tr> <td>a. Name of Subcontractor(s)</td> <td>b. Address (include Zip Code)</td> <td>c. Subcontract No.(s)</td> <td>d. DAR "Patient Rights" Clause No.</td> <td>e. Description of Work to be Performed Under Subcontracts</td> <td>f. Subcontracts Dates (yymmdd) Award</td> </tr> <tr> <td>None</td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>						a. Name of Subcontractor(s)	b. Address (include Zip Code)	c. Subcontract No.(s)	d. DAR "Patient Rights" Clause No.	e. Description of Work to be Performed Under Subcontracts	f. Subcontracts Dates (yymmdd) Award	None													
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<p>7. Certification of Report by Contractor/Subcontractor (Not Required if <input type="checkbox"/> Small Business or <input type="checkbox"/> Non-Profit Organization.) (Check Appropriate Box.)</p> <p>c. I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions", that such procedures have been followed and that all "Subject Inventions" have been reported.</p> <p><i>John C. Petersen</i> John C. Petersen, Director of Technology Licensing</p> <p><i>John C. Petersen</i> John C. Petersen, Director of Technology Licensing</p> <p>97/10/14 Date (yymmdd)</p>																									